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TITLE: A Dietary Strategy to Maximize Bone Mass in United States Naval Academy Midshipmen

PRINCIPAL INVESTIGATOR: Mona S. Calvo, Ph.D.

CONTRACTING ORGANIZATION: U.S. Food and Drug Administration
Rockville, Maryland 20852

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Mona S. Calvo, Ph.D.

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U.S. Food and Drug Administration
Rockville, Maryland 20852

E-Mail: mona.calvo@cfsan.fda.gov

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13. ABSTRACT ***(Maximum 200 Words)***

The study evaluates the efficacy and safety of two different types of dietary interventions to promote gain in bone mass at several skeletal sites and changes in hormonal or bone marker levels indicative of bone accretion in young Naval Academy Midshipmen. The dietary interventions optimize different nutritional factors, not just calcium intake, and enable us to examine the effect of maximizing all the nutrients essential for both bone matrix formation and bone mineralization under conditions of usual dietary intake at the Naval Academy. The supplementation trial was initiated in March 2000 in 161 Midshipmen from the classes of 2002 and 2003 who under went all required baseline-screening procedures. After sampling the supplements, these 161 Midshipmen agreed to participate for the two-year duration. The Midshipmen were randomized according to BMI and gender to one of four different dietary groups, each of which consume a unique combination of calcium supplement or placebo, and either a fortified protein bar or its placebo. The supplements are to be consumed every day for the study duration. After six months of taking the supplements, 78 Midshipmen remain in the study and 25 of the remaining participants are women. Six-month blood, urine and dietary records are currently being collected. Many of the baseline parameters have been analyzed and some will be analyzed with the six -month collections. Despite the very high rate of attrition, the four group sizes remain similar with 18 to 20 subjects per group. We have also persuaded 25 subjects, who dropped out because they no longer wanted to take the supplements, to continue in the study as sentinels. By providing bone, blood and dietary information, they will allow us to compare the supplemented and placebo groups to those consuming the usual Naval Academy diet.

14. SUBJECT TERMS : Peak Bone Mass, Calcium Supplements; Food-based Vitamin and Trace Mineral Protein Supplement; Bone Mineral Density; Bone Turnover Markers; Parathyroid Hormone, Insulin-like Growth Factor

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INTRODUCTION

The overall goal of this study is to maximize the amount of bone formed before skeletal maturity is achieved in the late twenties in order to reduce stress fractures during physical training and the risk of osteoporotic fracture later in life. Low bone mass is the strongest risk factor for bone fracture and early adulthood is considered the last window of opportunity to maximize gain in bone mass. The objective of this study is to optimize nutritional factors that would provide the needed building blocks and biochemical tools for maximal gain in bone mass during early adulthood. Two types of dietary supplements are used to promote gain in bone mass in physically active Naval Academy Midshipmen. We examine the effect of optimizing either calcium intake alone or in combination with adequate intakes of other nutrients essential for building bone. Many of these other essential nutrients are limited in the typical diets of teens and young adults, particularly when they leave home and enter college. We compare gains in bone mass at several skeletal sites, changes in urinary loss of calcium, serum levels of calcitropic hormones, and changes in markers of bone turnover in four groups of male and female Midshipmen. Each of the four groups consume different combinations of calcium supplements or placebo tablets, both with a nutrient fortified protein supplement or its placebo. Both types of supplements are to be consumed over two years. If optimizing the diet is effective, we would anticipate greater changes in bone mass or changes in markers of bone turnover and calcitropic hormones, and lower levels of urinary calcium excretion. All of these endpoints are indicative of bone accretion for groups consuming any combination of the dietary supplements. This would be in contrast to little change anticipated in these parameters for the placebo/placebo-fed Midshipmen consuming only their usual diet at the Naval Academy. We focus on the freshman and sophomore classes, which contain younger Midshipmen with the greatest overall potential for increasing bone accretion through diet and exercise. By monitoring changes in the calcitropic hormones, insulin-like growth factor, and in resorption and formation markers of bone turnover, and changes in urinary loss of calcium, we will gain insight into the possible mechanisms through which an optimal diet may influence gain in bone mass in physically active young adults.

Body

Tasks listed in the Approved Statement of Work that are pertinent to the Annual Report and covers research for the period of September 30, 1999 to September 29, 2000 are as follows:

Task 1 Months 1-8, Negotiate contracts for test products and their analysis, begin recruitment of men and women with testing and baseline measures needed to stratify to treatment. Secure GLP procedures for manufacture of test supplements: assure that products meet FDA specifications. Initiate ORISE personnel on study.

Task 2 Months 9 -12, Stratification of volunteers to treatment and treatment initiation.

Task 3 Months 13-18, First 6 month blood and urine samples and second 3 day food record. All collections gathered prior to assay for specific endpoints. Spontaneous compliance checks conducted.

Below, I describe the research accomplishments, progress, and various problems associated with each of the elements in Tasks 1, 2 and 3 of the approved Statement of Work. As a needed point of clarification, the months identified in the Statement of Work do not describe time since the award of the grant, but rather the time frame of the feeding study which was adapted to coincide with the academic schedule of the U.S. Naval Academy. We designed the study to start feeding the dietary supplements after the Midshipmen had completed their first semester at the Naval Academy, because the largest attrition usually occurs at the end of the first semester.

Task 1:

Month 1-8, Negotiate contracts for test products and analyses, and secure GLP procedures for manufacture of test supplements; assure that products meet FDA specifications. Initiate ORISE personnel on study.

Towards this task, FDA successfully negotiated an Inter- Agency Agreement (IAG) with the Combat Feeding Program at Natick Soldier Center, Natick, MA. To date, we have received the entire shipment of calcium supplements and placebo capsules needed for the study duration and a total of 90,000 placebo and test bars (fortified protein supplement and placebo). The Combat Feeding Program is simultaneously conducting quality assurance testing to meet FDA specifications for the protein/placebo bars and has completed these tests for the calcium supplements and placebo capsules. In response to our request for a third flavor of the test and placebo bars, the Combat Feeding Program very rapidly developed a new flavor, chocolate, which gives the Midshipmen a much needed variety of flavors: apple-cinnamon, cran-

raspberry and chocolate. We believe that this effort will help to quell the monotony of consuming these supplements every day. The new flavor was introduced in September wh the Midshipmen returned from summer sea duties. Th combat Feeding Program's outstandi performance to develop the new flavor that met the required specifications of the test produ and placebo in such a short period of time is highly commendable and their efforts merit recognition.

To assure adherence to good laboratory practices and safety measures, any analyses conducted at FDA facilities must file Form 3244 for approval of each study conducted at CFSAN. This approved application of the study protocol and safety procedures that must b closely followed in conducting the necessary analyses of blood and urine in this study are presented, in part, in the Appendices pages 11 to 19.

With respect to negotiating contracts for the analyses for insulin-like growth factor and the bone turnover markers, osteocalcin, bone specific alkaline phosphatase and N-telopeptide of type 1 collagen of bone will be carried-out through sole source contracts that were negotiated with Dr. Clifford Rosen at the University of Maine and Dr. Caren Gundberg at Yale University Medical School as shown in their respective contracts presented in the Appendices on pages 20-27 and pages 28-32.

The FDA offered an *Inter Agency Agreement* to the Office of Naval Research to fund a third-party arrangement for the determination of bone mineral density scans on the participating Midshipmen that would be supervised by Dr. David Armstrong, Co-investigator and employee of the Henry Jackson Foundation. This IAG was initially rejected by the Office of Naval Research in 1999, and later accepted in early 2000 as shown in the Appendices on pages 33-36. However, this has not proved to be the best mechanism to fund baseline, year one, and year two bone and total body scans and anthropomorphic measures on the Midshipmen. While FDA was notified on September 28, 2000 that the Office of Naval Research did intend to honor the IAG, baseline scans and anthropomorphic measurements have not been delivered, as yet. LTC. Karl Friedl and Dr. Stephen Grate have been apprised of these problems in securing the baseline information and are working towards their resolution. ***The results of the baseline scans were secured in April 2001 and are reported in the Appendices on pages 37–43 on this revised annual report.***

ORISE personnel initiated on study .Ms. Sheila Mackertich, R.D., L.D. was secured through ORISE and was initiated on October 18, 1999 as the resident Study Coordinator located on site at the Naval Academy in Annapolis, MD. As Study Coordinator, she recruited Midshipmen, arranged for baseline bone scans, provided individual instruction on the basics of dietary records using food models, arranged for 24 hour urine collections and 3-day dietary records, coordinated and contacted Midshipmen for appointments for blood collections and set-up and supervises the blood and urine collection process and delivers them to the CFSAN facilities in Laurel, MD for processing and analyses. Ms. Mackertich coordinates all study activities through the Academy's Public Affairs Office and Medical Clinic. In

preparation for blood draws, she coordinates activities and arrangements with staff in the Academy's Physical Therapy Unit and with the Food Services Unit so that food and juice are provided to the Midshipmen when their blood is drawn between 5:30 and 6:30 AM. She analyses all dietary records and initiated an internal survey to determine the level and type of beverage consumption in the Midshipmen (See Appendices pages 44-48). This was in response to a growing concern that young adults are consuming more carbonated soft drinks and less milk, a dietary pattern that was recently linked in several studies to increased incidence of bone fractures in teens and adolescents. Ms. Mackertich also distributes the dietary supplements to the Midshipmen and monitors their compliance. She has generously made herself available for dietary counseling and the Midshipmen take advantage of her skills and concern for their general well being. To bolster enthusiasm and promote retention of the subjects, Ms. Mackertich designed a T-shirt with the logos of the participating agencies (CFSAN/ FDA, USNA, and the Army Combat Feeding Program, see pages 49-50 of the Appendices, and some appropriate messages that build team spirit and support . These have been ordered and should be available for the Army / Navy football game, December, 2000.

TASK 2:

Month 9-12.

Begin recruitment of men and women with testing and baseline measures needed to stratify to treatment.

We began recruiting male and female Midshipmen with the initiation of the class of 2003 in July 1999, but poor response from the freshmen class required us to recruit from the sophomore class as well. We obtained informed consent from 254 Midshipmen prior to taking any baseline measurements of bone density, 24-hour baseline urine collection, 3-day food records and baseline blood draws. The baseline sampling and bone scans were completed in March 2000, at which time 161 Midshipmen had completed all four of the baseline procedures (bone scans, blood sample, diet record, and 24-hour urine) and were willing to start the two year dietary supplement trial. These 161 Midshipmen were randomized based on their BMI and gender to groups who consume either a calcium supplement or a placebo bar and either a vitamin / mineral fortified protein and energy bar or its non-fortified placebo bar. Only Dr. Curtis Barton, Co-investigator and study biostatistician, knows the identitiy of the supplements consumed by each group as this is a double-blind study. Appendices page 51 shows the protocol instructions to be followed for taking each supplement and color identification for the supplements on each team.
With only 161 subjects recruited and starting the study, we fell very short of the desired 254 total Midshipmen to start the study. The 161 Midshipmen began taking the supplements in mid-March of 2000, which allowed them some time to acclimate to the routine before summer leave in late May. As the majority of Midshipmen leave the Academy in May for sea duty, they were provided with a four-month supply of supplements and bars in early May to carry them over. Upon their return

to class in September, a substantial number decided to drop out of the study. A variety of reasons were given to explain their decision to not continue in the study. These explanations included mandatory separation from the Academy for poor academic or physical performance, inability to transport the supplements aboard ship due to small locker space, or to added stress from participating in the study or fatigue from taking the supplements.

As of October 30, 2000, a total of 78 Midshipmen remain in the study, of these 25 are female Midshipmen. Fortunately, the 78 are equally distributed to all four groups with 18 to 20 Midshipmen per group. We established a sentinel group that will give us insight into the changes in the monitored parameters in Midshipmen only consuming the usual diet at the Naval Academy. This group consists of 25 of the Midshipmen who dropped out of the study during the summer, but agreed to continue the blood, urine, and diet collections every six months and yearly bone scans. The greater than 50% attrition rate suffered after the first six months of supplementation is not altogether surprising given that the greatest per cent loss was observed in Midshipmen recruited from the freshman class, who are clearly under the greatest stress. Other studies have found that this age group is probably the most difficult to retain in short or long duration studies that are only observational because of the many competing demands made of them, which is only compounded in the first year at an elite military academy.

Task 3:
Months 13-18,

First 6 month blood and urine samples and second 3 day food record. All collections gathered prior to assay for specific endpoints. Spontaneous compliance checks conducted.

We started our 6- month blood, urine, and dietary data collections in September 2000 and anticipate finishing the last segment which include the 24 hour urine collections and dietary data in November 2000. Clinical chemistry analyses have been performed on baseline and on the first six month samples and are currently being compiled. Analyses of some baseline hormones are complete and some will be processed together with the 6 month samples. The serum and urine samples for bone turnover markers will be sent for simultaneous analyses of baseline and 6 month samples, as will the serum samples for IGF-1 and its binding protein. Baseline dietary records have been analyzed for estimates of nutrient intake. The baseline nutrient intake data for the Midshipmen who completed the 3-day dietary record was the subject of an abstract recently submitted to the Experimental Biology 2001 meetings (Appendices page 52).

Key Research Accomplishments

- There are no key research accomplishments to date as the work is in the first six months of a two year dietary interventions. These samples are in the process of being collected and analyzed.

Reportable Outcomes

- In this early phase, there is only one reportable outcome shown in Appendices page 53. This abstract assesses baseline nutrient intakes in Midshipmen focussing on those nutrients critical to building and strengthening bone. The abstract compares these findings to the current Dietary Reference Intakes for the Midshipmen's age and sex group and to a group of comparable young adults participating in the NHANES III survey. The later provides a comparison to nationally representative nutrient intakes in 17 to 20 year old young men and women.

Conclusions

- No conclusions can be made in this early stage of experimentation.

References

- There are no reportable references at this time.

Appendices

Documents **Pages**

15HQ-001-Z

. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration

CFSAN STUDY PROTOCOL

Note: Form FDA 3244 must be completed for approval of each study conducted in CFSAN (GLP and Non-GLP).

Complete the following questions, any which do not apply respond by entering N/A, do not leave any space blank.

Classification of Study: GLP ☐ NON-GLP OTHER- Clinical Investigation

1. Protocol Title:

Analyses of Human Serum and Urine for Electrolyte, Calcitropic Hormone, Vitamin and Mineral Levels.

This protocol is in support of a CFSAN clinical investigation funded by the Department of the Army entitled " A Dietary Strategy to Maximize Bone Mass in United States Naval Academy Midshipmen".

2. Facility (name and address):
MOD-I Facility, room 2412, 8301 Muirkirk Road Laurel, MD 20708

3. Study Objective (if additional space is required, use 8" x 11": To process and store blood and urine samples and analyze them for specific levels of calcitropic hormones, electrolytes, and vitamins and minerals. These end measures are all needed by the clinical investigation named above to assess response to dietary intervention. The specific objectives of this clinical study are presented in the FDA Research Involving Human Subjects Committee (FDA RIHSC) approved protocol presented in **Appendix A.**

4. Study Dates:

a. Proposed Date Study Initiated:
Protocol Initiated February, 2000

5. Name of Study Director:

b. Proposed Date Study Completed:
February, 2003

6. Name of Principal Investigator (for NON-GLP Studies): Mona S. Calvo, Ph.D.

7. Organizational Unit for the Study Director (i.e., office, division, branch): Office of Nutritional Products, Labeling and Dietary Supplements, Clinical Research and Review Staff (no branch)

BFQ NUMBER: BFQ-001-Z

Department of Health and Human Services
Public Health Service
Food and Drug Administration

CFSAN—AMENDMENT TO PROTOCOL

Classification of Study: GLP ☐ NON-GLP ☐ Clinical Investigation

1. Protocol Title: Analyses of Human Serum and Urine for Electrolyte, Calcitropic Hormone, Vitamin and Mineral Levels

2. Type of Change: (✔ one)
Correction ☐ **Addition** Deletion

3. Section of Protocol Amended: Appendix B Experimental Design

4. Reason for Amendment: To document the disposition and use of human serum remaining after obligation to study needs are met and remaining plasma whole blood sample after hematology analyses by the Clinical Chemistry Staff. There are two objectives to this amendment. One is to establish the use of pooled excess sera to serve as an internal assay control for all analytic methods using serum for the duration of the study. This means that approximately 200 one-ml aliquots must be generated and stored in the ultra cold freezer for use as an internal standard in each assay. There needs to be sufficient number of human serum samples to carry through the entire 2.5year study duration without subjecting the internal control to freeze/ thaw action that usually alters analyte levels. This will also allow better assessment of assay performance (coefficient of variation) from assay to assay over this time period and within the same assay for RIAs.

The second objective is to transfer back to the Study Director the remaining serum not used by the Clinical Chemistry Unit staff in measuring the clinical parameters described in *Quantification of Specified Clinical Parameters in Human Serum, Urine, and Specified hematological parameters in Human Whole Blood for the Study entitled "A Dietary Strategy to Maximize Bone Mass in United States Naval Academy Midshipmen"*. I addition, the Study Director will transfer the remaining plasma whole blood sample to the Clinical Chemistry Staff for purposes of training ~~newer staff members~~. more Human plasma sources are difficult to obtain making this an ideal opportunity for instruction with a large number of human plasma samples.

5. Description of Change:
Addition: Excess serum is pooled from day to day during the period of blood sampling until approximately 200 ml of serum are obtained. This pool is held at 4 degrees during the entire collection process and not frozen. The pooled serum is mixed for 20 minutes on ice and then aliqoted to one-ml vials labeled as internal controls and frozen in the ultra cold freezer. Each sample will only be used for one assay and never refrozen for repeated use.

Transfer of the remaining serum used by the Clinical Chemistry Unit back to the Study Director will be achieved using BFQ-001-Z form B (attached). Upon receipt this sample will be stored frozen in the Ultra cold freezer for use in assays that are less sensitive to freeze thaw disruption.

~~No~~ Original will be used more transfer form ~~is required~~ to make permanent transfer of the human plasma samples from the Study Director to the Clinical Chemistry Staff since revised CCU Record Form #1.Rev.A was used to transfer the samples initially. The Clinical Chemistry Staff will file a statement describing how they will be used for instruction. No subject's name or identity is transferred with these samples.

c. Proposed Statistical Methods: Dr. Curtis Barton of OSAS, CFSAN, FDA will conduct all the statistical analyses using SAS statistical software. A repeated measures analysis of covariance model will be used to analyze each response variable, with baseline measurement as the covariate. Other individual characteristic such as Midshipmen height, weight or race may be included as covariates as well. The between subject factors are calcium intake, mineral and vitamin intake, and sex. Each of these factors has two levels. The within-subject factor is duration of treatment, with two levels (12 and 24 months). All main effects and interactions will be tested using a two-tailed significance level. Data will be examined for satisfaction of the assumptions of a Gaussian distribution and homogeneity of variance-covariance matrices. Refer to pages 13 to 14 of the attached approved protocol in Appendix A for estimates of statistical power.

d. Records to Be Maintained:

The study director, Dr. Calvo, is responsible for maintaining all data records for analyses conducted at the MOD-I Facilities. This is a double blind randomized study with only Dr. Barton having knowledge of the identity of each treatment group. All patient data is linked to an identity code (UPI #) assigned to each Midshipmen after obtaining informed consent. All samples are identified by the subject identity code, the nature of the sample (e.g., serum, plasma, urine or red blood cells) and the date or other indicator for the collection period of the sample, such as baseline or 6th month. No sample or analytical value will be linked by name to any Midshipman participating in this study, only through their identity code.

Other records to be maintained by the study director, Dr. Calvo, include all those necessary to demonstrate adequate and appropriate function of critical equipment needed in the processing and analyses of these samples. These include the following:

Equipment Maintained	Frequency of Recording
1. Temperature logs on the laboratory Refrigerator/Freezer	~~a minimum of weekly, trying for~~ when in use daily record
2. Balances logs and weight calibrations	each day of use, balance will be calibrated
3. Automatic pipette calibrations	calibrated every six months and periodically as needed
4. Refrigerated centrifuge; Spectrophotometer	balanced and serviced yearly; serviced yearly
5. Eye Washing Device	flushed weekly

e. **Safety Precautions or Hazard Identification:** Safety procedures described in CFSAN's manual for Bloodborne Pathogen Exposure Control Plan (1999) are followed at all times in processing and analyzing human blood and urine samples. Hazard identification signs are placed on the Lab 2412 door and on all containers that will be used to transport blood tubes from the place of origin, U.S. Naval Academy, to the MOD-I facility. The secondary container used to transport the blood are leak resistant coolers, that clearly display the BiHazard Warning signs. No eating of food or drinking of beverages is allowed in the laboratory where the blood and urine samples are processed. Latex gloves, a laboratory coat and corrective safety glasses are worn by the study director at all times during the processing of the urine and blood samples for storage or during analysis. Biohazard boxes and red bags, and sharps containers are placed and used at both the site of the blood draws, U.S. Naval Academy in Annapolis, and in Lab 2412 where blood is centrifuged and serum and plasma are aliquoted and stored for later analyses. The urine volume is measured and aliquots are saved and stored for later analyses with the excess urine discarded directly down the laboratory sink and flushed repeatedly with water. Empty urine containers are rinsed initially and then scrubbed with a veterinary grade disinfectant and rinsed repeatedly with very hot water on the same day that the urine samples arrive at MOD-I. The area surrounding the sink, the volumetric glassware used to measure urine volume and the plastic tubs transporting the urine jugs are swabbed down with the same disinfectant at the end of processing. These urine samples are stored in nalgene bottles in plastic lined boxes at -20^0C in the second floor walk-in freezer. After centrifugation and aliqotting of serum, plasma, or red blood cells to the appropriate storage tubes will be conducted under the fume hood. The vacutainer tubes and caps are discarded in the sharps containers and red bags, respectively. Protective plastic lined paper will cover the main work area for blood processing and will be discarded at the end of each day with other disposable apparatus used to process the blood samples (plastic pipettes, etc.). The hood area will then be wiped down with disinfectant (1:100 dilution of Chlorox) The blood-derived samples will be stored in skirted screw cap mini-vials in freezer storage racks at -80^o C in the freezers located near the second floor cold room (Biohazard Label will be Placed on the Door). Radiation safety procedures will be amended to the protocol when application is made to purchase the specific radioimmunoassay kits for hormone determination.

e. Appendixes (If not applicable, indicate N/A or □See Attached□. Assign alphabetical or numerical identification to attachments):

Appendixes A, B and C are attached.

(1) Pathology Protocol: N/A

(2) Analytical Chemistry Protocol: N/A

(3) Clinical Chemistry Contract\Protocol: **Attached in Appendix C.**

(4) Hematology Contract: **Attached in Appendix C.**

(5) Other (Specify): N/A

BFQ NUMBER: OO1-Z

Department of Health and Human Services
Public Health Service
Food and Drug Administration

CFSAN—AMENDMENT TO PROTOCOL

Classification of Study: GLP ☐ NON-GLP Other: Clinical Investigation

1. Protocol Title: Analyses of Human Serum and Urine for Electrolyte, Calcitropic Hormone, Vitamin and Mineral Levels.

2. Type of Change: (✔ one)
Correction X Addition
Deletion

3. Section of Protocol Amended: Section 11. B. Radioactive materials and g. RSC approval for use of Radioactive materials.

4. Reason for Amendment: As indicated in the original study protocol, we are submitting in this amendment the explanation for

the safe use of radioactive materials and the approval to use these materials from the Radiation Safety Office.

5. Description of change (see below)

6. Signature of Study Director 7. Date

Description of Change: The following Protocol is submitted for use of Radioactive materials.
Use of Radioactive Material. The protocols (kit inserts) for the hormone and vitamin assays involving radioactive materials are presented in Appendix B of the CFSAN Study Protocol for BFQ-001-Z. Below we list the amount of radioactivity contained in one radioimmunoassay kit for each of the 5 specific assay kits. The last column of the table estimates the total amount of radioactivity from ^{125}I that will be used to assay all the serum samples in one of the 5 total blood draws. The blood draws are made at baseline and at 6 month intervals thereafter for 2 years. Each blood draw generates approximately 200 subject samples in duplicate to be analyzed. We anticipate that the total radioactive material use for an entire year will be approximately 400 μCi ^{125}I with an average use of 5 μCi ^{125}I/day with all except one assay which uses 20 μCi ^{125}I/day. These estimates place this protocol in Hazard Class IV, Type C for possession limits > 1 ALI which is 40 μCi ^{125}I for Ingestion and 60 μCi ^{125}I for Inhalation.

Assay Kit to be used	Isotope	No. Samples/kit	Radioactivity/kit	Total radioactivity used per year
25-hydroxyvitamin D	^{125} Iodine	40 samples	4 μCi	40 μCi
1,25 dihydroxy vit D	^{125} Iodine	40 samples	5.5 μCi	55 μCi
Ferritin	^{125} Iodine	40 samples	4 μCi	40 μCi
Intact PTH (IRMA)	^{125} Iodine	40 samples	20 μCi	200 μCi
Folate and vitamin B_{12}	^{125} Iodine	40 samples	4.5 μCi	45 μCi

Total anticipated annual use: 380 μCi

Handling of radioactivity. For all the assay kits above the proteins or beads that are radioiodinated do not have to be reconstituted.
The tracer additions may be made to the assay tubes using a calibrated automatic pipette. The pipette tip and empty radioactive stock bottle containing the tracer are disposed of in the dry radioactive waste bucket.
Amount of activity used per procedure. The activity associated with each kit is shown in the above table. A continuous Inventory and record of disposition of the radiotracer from each kit will be kept. For each kit, we will record (inventory) the total amount of activity dispens, the dates dispensed and the status to date of the dispensed portion. Radiotracer from

similar assays kits will not be mixed, so each stock bottle will be appropriately disposed of at the end of each assay which will entail returning the inventory form to RSO and notification of the RSO for pick up of dispensed or unused vials.

Storage and Use sites. All the kits will be stored briefly after receipt in the refrigerator located in Laboratory 2412 of the MOD I Facilities. The analyses using these kits will be conducted in the fume hood of this laboratory and will involve the adjacent clinical Centrifuge and near laboratory bench area. These are indicated on the attached diagram of the laboratory, 2412. Daily, after use of the radioactive materials the laboratory area where the isotopes are used, the work area will be surveyed with a suitable survey meter provided by the RSO. The Form attached at he end of this amendment outlines the information that will be recorded with each routine use survey and for each comprehensive contamination survey of wipe tests which are required on a weekly and quarterly basis for ^{125} Iodine. Each survey report will include: the individual performing the survey; a diagram of the area surveyed with the locations of the specific swipe tests and or frisk; a map indicating equipment used and surveyed; specific information on the survey meter used, (manufacturer, model and serial number); background levels in dpms, diagram of the position of any spills and the corrective action taken to decontaminate.
The fixed contamination limit is 500 dpm/100 cm^2 for all radiation types. A major spill is considered $>100_{\mu}$Ci or $>$ 1000 ml which will not be reached in using any of these kits, thus only minor spills could be anticipated with the proposed use shown in the table above. Radioactive clean-up kit is stored in the laboratory with easy access under the sink and absorbent-plastic backed paper or absorbent pads is used in all work areas to facilitate spill containment. Eye wash and safety shower are located less than 25 feet from the work area as indicated on the survey diagram. The RSO will calibrate the survey meter each year. Comprehensive surveys are conducted monthly in which a larger number of swipe tests and a more extensive area of the laboratory is surveyed will be conducted throughout the period of active use of radioactive materials. Prolonged periods of inactivity for the radioactive materials use will be recorded in the laboratory log of radiation safety along with records/outcomes of all surveys. The attached audit form (BFQ-001-Z radiation safety audit Form will be used with daily surveys as well as the more comprehensive surveys as the mechanism of recording outcomes including spills and the results of spill clean-ups and evidence of decontamination.
Comprehensive swipe surveys will also be conducted for the gamma counter and surrounding area on a monthly basis.
For all swipe surveys, the survey results will be recorded with the survey and the questions posed on the form answered.
Mechanism used to store waste. Liquid waste will be housed in the storage vessels supplied by RSO. This will be labeled appropriately with caution tape and radioactive waste materials tags indicating the isotope, the final pH which must fall in the range of 6-10 because all the liquid will be aqueous soluble. Only one source of radioactivity will be used in the l ab, ^{125} Iodine, which has a half life of 60 days and can be stored by the RSO with other second stream isotopes (30-60 day T1/2) until it has
decayed through 10 half lives.

19. Experimental Design

a. Narrative Discussion of the general experimental plan including method for control of bias.

This narrative only describes the protocol design for that segment of the clinical study conducted at the MOD-1 facilities. Information concerning the overall study design is presented in its briefest form in the consent form and in greater detail in the approved protocol in Appendix A.

Upon entry into the study and at 6 month intervals thereafter for the study duration of 2 years, blood is drawn and urine is collected for routine laboratory testing of electrolytes, hematology measures, calcitropic hormone activity, and end measures of vitamin and mineral nutritional status. For each of the five total blood collections 120 to 150 cc are drawn in the morning before breakfast (fasted state) and a "spot" urine sample is obtained from approximately 40 Midshipmen per day. This process is repeated on weekdays only until samples have been obtained from all of the anticipated 240 participating Midshipmen. Blood and urine samples are collected between 0500 and 0630 hours at the Naval Academy in Bancroft Hall where all the U.S. Naval Academy Midshipmen reside. The samples are immediately transported to the MOD-I facility where they are processed as described below. Dr. David Armstrong, the Study Director at the U.S. Naval Academy and a licensed phlebotomist will oversee the blood draws. Mrs. Sheila Mackertich, R.D. the Study Coordinator will oversee the collection of spot urine samples and will transport the blood and urine to MOD-1 for processing and analyses by the Study Director, Dr. Mona Calvo. A modified version of the CCU record form #1 with minor modifications attached will be used to transfer all samples from the Naval Academy to the Study Director at the MOD-I facilities (attached).

Medical waste generated at the Naval Academy will be disposed of at this site to avoid transporting it back to MOD-1. The filled sharps containers and other waste will be bagged in red biohazard bags and boxes and will be disposed of as infectious waste through the U.S. Naval Academy Clinic who routinely handles the waste from standard blood draws from hundreds of Midshipmen.

Blood Samples:

Whole blood samples are presented to the Clinical Chemistry Staff for analyses of the specific hematology endpoints described below that are best measured within 24 hours of sampling. The rest of the processing involves centrifugation of the blood to yield serum or plasma or blood cells which are aliquoted into multiple mini storage vials and frozen at -80^0C for future analysis. The number of storage vials generated for each subject is dependent on the number of vacutainer tubes successfully filled. The goal is to have a dedicated vial for each of the hormonal, vitamin and mineral analytes to obviate loss of activity with multiple freeze-thaws.

The Table below summarizes all the analytes to be measured, the specific blood tube required for best measurement of the analyte and the method to be used to measure the analyte. When available the assay information presented in kits are referenced and attached at the end of this section. The protocol or SOP for processing the two types of vacutainer tubes is also attached at the end of this section. The serum and plasma will also be used for analysis conducted outside of MOD-1 by scientists collaborating on the clinical investigation. These parameters include the bone markers, osteocalcin, undercarboxylated osteocalcin and NTx, the hormone Insulin-like growth factor 1, and indicators of vitamin K status, serum phylloquinone, and dihydrophylloquinone. If sufficient quantities of plasma remain after conducting these essential analyses and funds are available, measurement of parameters indicative of folate and vitamin B_{12} status including methionine, homocystine, folacin and vitamin B_{12} analyses will be carried out at MOD-1. In this event, appropriate amendments will be made to the study protocol. However, it is important to note that no additional blood draws will be made for these analyses, as we will rely only on the availability of stored serum and plasma.

Analyte to be measured	Vacutainer Tube	Minimum volume needed for measurement	Method of Analysis	Party Conducting the Analysis
Intact Parathyroid Hormone	Serum, Red top	500 µl (2 ml vial)*	IRMA** using Diasorin N-tact PTH SP kit	M.S. Calvo Kit procedure will be amended when available.
25- Hydroxy Vitamin D	Serum, Red top	150 µl (1ml vial)	RIA**, double antibody using Diasorin Kit for 25-Hydroxy vitD	M.S. Calvo Procedure, Kit instructions attached.

1,25 Dihydroxy Vitamin D	Serum, Red top	2.25 ml (2, 2ml storage vials)	RIA**, double antibody using Diasorin Kit for 1,25-Di Hydroxy Vitamin D	M.S. Calvo Kit procedure attached.
Ferritin	Serum, Red top	150 µl (1 ml vial)	IRMA** using Diasorin assay kit for ferritin	M.S. Calvo Kit procedure attached.
Transferrin Receptor	Serum, Red top	150 µl (1 ml vial)	EIA*** Using the Ramco Laboratories Kit	M.S. Calvo Kit procedure attached
Clinical Analytes: Sodium Potassium Chloride Calcium Phosphate Magnesium BUN Creatinine Alkaline phos. Total protein Albumin Uric acid TIBC Iron HDL Tg LDL (calculated) Cholesterol	Serum, Red top	2 ml (store a minimum of 4 ml)	Dimension Ar Using CCU-SOP No. 2-rev.2 See CCU Contract/Protocol in Appendix C	Clinical Chemistry Unit Staff
Hematology : WBC RBC HgB Hct MCV MCHC RDW PLT MCH MPV	Plasma K3 EDTA 5 ml Lavender top tubes	0.75 ml whole blood for hematology (no whole blood will be stored)	Coulter S+IV Using CCU-SOP No.28-rev.7. See CCU Contract/Protocol in Appendix C	Clinical Chemistry Unit Staff

* Indicates desired amount (shown in parentheses) of serum or plasma to be stored to meet assay demands for repeats and duplicate measures.
** RIA, radioimmunoassay, or IRMA, immunoradiometric assay, all use ^{125}I as the radioactive tracer.
*** EIA, enzyme –linked immunoassay, does not involve radioisotopes.

Processing of Vacutainer tubes:

Plasma and whole blood tubes: All tubes are gently mixed after filling. For whole blood use, such as for hematology measurements, the analyses must be completed within 24 hours of collection.

For plasma used in phylloquinone assay and potentially to analyze homocysteine and methionine levels, the tubes are placed on ice after drawing. Lavender top tubes designated for phylloquinone assays are protected from light after drawing by wrapping the tube in aluminium foil. Lavender tops designated for these assays are transported on ice in a cooler and centrifuged at 4^0 C with caps on for 10 minutes at 3000 rpm and the plasma fraction immediately

separated from the red cells. For the tube used in the phylloquinone assay, equal plasma volumes from one tube are transferred to two appropriately labeled opaque storage vials protected from light and immediately frozen and stored in the ultra-cold freezer. The plasma tube to be used in the methionine/homocysteine assay is transferred to one vial and store frozen in the ultra-cold freezer. Plasma / whole blood samples for hematology measures are not centrifuged and are transferred in their original containers to the Clinical Chemistry Unit. Approximately 40 sample transfers are anticipated each day and no more than 60 samples will be transfered on a given day. A modified version of the CCU record form #1 with minor modifications attached will be used to transfer all samples from the Study Director to the CCU.

Serum blood tubes: Red top serum tubes are gently inverted five times and then allowed to clot for a minimum of 30 minutes at room temperature, but not to exceed two hours. After clotting the tubes are centrifuged at 4^0 C for 15 minutes at 760 x g, and then the serum is immediately separated with a disposable pipette and transferred into screw-cap mini storage vials.

All serum and plasma that is not assayed the same day of collection will be processed and immediately frozen for future analyses. These are stored in the second floor ultra cold freezers in labeled polypropylene tube racks. Care will be taken to record the freezer identification number on the temperature log in order to maintain consistent records

Urine Collections:

The collected spot urine is poured into a small labeled plastic bottle and brought back to MOD-1 and frozen at –20°C for future analysis at a contract laboratory.

On a separate day from the blood and spot urine collection and at baseline and at 6month intervals over two years, 24 hour urine collections are made. Each Midshipmen receives written
And verbal instructions on how the urine is to be collected (see attached sheet). Participants are provided with a large Nalgene collecting jar containing 20 ml of 6 N HCL and urine is collected precisely as described in the attached procedure in Appendix A. Midshipmen are verbally cautioned about the acid in the jar and a written warning is also given in the procedure. The urine is returned to MOD-1 and the information sheets are used to evaluate completeness of the 24-hour collection. Total urine volume is carefully measured and recorded using graduated cylinders and three aliquots are taken and stored in labeled bottles at -20^0 C until assayed. One aliquot is assayed for calcium, creatinine and phosphate by the CCU staff using the Dimension AR and CCU –SOP No. 2-rev. 2.
Due to the require acidification of the urine with acid, the Dimension AR cannot be used to accurately measure sodium and potassium levels in the 24 hour urine collections. With respect to these needed measures we are in the process of determining an acceptable alternative method to use on acidified urine samples. When this is determined, the amendment to the protocol will be submitted. The urinary sodium and potassium analyses will be carried out by the CCU Staff or by a suitable contractor depending on the availability of the equipment needed for these analyses.

b. Types and Frequency of Tests, Analyses and Measurements: (Refer to the Sop's associated with each.) Note: Schedule for Critical Phases Are to Be Added by "Amendment to Protocol" After Protocol Approval and Before Initiation of the Study.

The types of analyses needed are discussed in detail in part a. Blood, spot and 24-hour urine collections will be collected as described at 5 different times over the two-year feeding study (at six month intervals). The exact times for these collections are based on the Midshipmen's rigid schedule and can not be provided in advance. Every effort will be given to coordinate this work with the CCU Staff allowing as much lead-time as possible under the circumstances. With an estimated 200 to 250 Midshipmen participating there will be an estimated 250 samples for each of the analytes described above with an anticipated total of 1,250 samples for each analyte over the two year period.

DEPARTMENT OF
HEALTH AND HUMAN SERVICES

PURCHASE/SERVICE/STOCK REQUISITION

REQUISITION NUMBER: X24830

OFFICE CODE/SYMBOL:

BPA and Call No. _____

TO: Division of Contracts and Procurement Mgmt. OFACS	REQUEST FOR: ☐ PURCHASE ☒ SERVICE ☐ STOCK ISSUE ☐ RENTAL/LEASE		
REQUESTING ORGANIZATION: Food and Drug Administration	CUSTODIAL AREA: CFSAN	DATE: 06-29-2000	OBJECT CLASS: 25.55
FOR REFERENCE CALL: Juanita Pointer	EXTENSION: 205-4098	APPROPRIATION: 7500600 22390D/10	
DELIVER TO: Mona S. Calvo, Ph.D. (HFS-452) Office of Special Nutritionals, CFSAN 200 C. Street, SW Wash., DC 20204		CAN: 0-6991697-X-24830 DATE REQUIRED:	

ITEM NO.	DESCRIPTION (INCLUDE STOCK NUMBER, MODEL/PART NO., ETC.)	QUANTITY REQUIRED	UNIT OF ISSUE	COST UNIT	COST TOTAL
	Services for Measurement of Changes in the Levels of Resorptive and Formative Markers of Bone Turnover as Evidence for the Efficacy of Dietary Intervention Strategies at the U.S. Navel Academy Period of Performance: July 31, 2000 July 31, 2002 Source: Caren M. Gundberg, Ph.D. Associate Professor Department of Orthopaedics and Rehabilitation Yale University School of Mediciane P.O. Box 208071 New Haven, CT 06520-8071 Phone: 203-785-7548			49,280.00	49,280.00

I certify that the property/services requested are required for Government business, and are not available from excess or current assets.*

FUNDS AVAILABLE (Signature/Title): for Barbara Gautreaux, Leader, BEFPT — DATE: 7/7/00

TOTAL: $49,280.00

REQUESTED BY (Signature/Title)*: Mona S. Calvo, Ph.D., Project Officer	DATE: 07/05/00	RECEIVING OFFICIAL - I certify that the quantities indicated in the "Quantity Required" column above have been received in total or as annotated.	
RECOMMEND APPROVAL (Signature/Title)*: Lori Love, M.D., Ph.D., Chief, CRRS, ONPLDS	DATE: 07/05/00	RECEIVING OFFICIAL (Signature/Title)*	DATE
APPROVED BY (Signature/Title)*: Christine J. Lewis, Ph.D., Act Dir ONPLDS	DATE: 07/05/00	ORDER NO. (PO, DO, FEDSTRIP, ETC.)	ORDER DATE
PROPERTY MANAGEMENT OFFICER (Signature)*	DATE	VOUCHER NO.	VOUCHER DATE

HHS-393 (Rev. 6/90)

Requisition Number: X-24830

Justification for Other than Full and Open Competition

1. **Identification of the Agency and the Contracting Activity:**

Agency: Food and Drug Administration
PHS/DHSS

Program Officer: Mona S. Calvo, Ph.D. HFS-805

Contracting Activity: Division of Contracts and Procurement Management HFA-512

2. **Nature of Action Being Approved:**

Full and open competition is restricted. The acquisition must be from a specified source:

Caren M. Gundberg, Ph.D.
Associate Professor
Department of Orthopaedics and Rehabilitation
Yale University School of Medicine
P.O. Box 208071
New Haven , CT 06520-8071
Phone: 203-785-7548

3. **Description of Supplies or Services and Estimated Value:**

Title: Measurement of Changes in the Levels of Resorptive and Formative Markers of Bone Turnover as Evidence for the Efficacy of Dietary Intervention Strategies at the U.S. Naval Academy.

Background:

In response to a proposal submitted to meet the objective of a Request for Proposals investigation strategies to improve bone health in military men and women, CFSAN was awarded funding from the Department of Defense to conduct a two-year dietary intervention study (IAG No. 224-98-2568). This study involves supplementing male and female Midshipmen from the United States Naval Academy with calcium and specific nutrients which have been identified as limited in the diet and essential to bone matrix growth and mineralization. The dietary supplements are intended to optimize all nutritional factors to allow maximum gain in bone mass or density in this last phase of bone growth. Because denser bones are stronger, the risk of osteoporosis and bone fracture in old age is in part determined by peak bone mass or the total amount of bone mass achieved at [illegible]

maturity. We know that calcium is important to gain in bone mass in young children, but we do not know its significance in young adults whose bones have achieved full linear growth. The goal of this study is to determine if optimizing the nutrient intake of all the micro-nutrients needed for bone growth effectively increases gain in bone mass in young adults as it does in growing children.

The efficacy of these dietary interventions to promote gain in bone will be assessed by monitoring changes in bone mineral density at baseline and at the end of the first and second year. This measure requires at least a year before discernible differences in bone mass can be detected and there is always concern that these changes may be difficult to detect due to the inherent problems with precision error of the bone scanning technology and the relatively low rate of gain in bone mass after puberty. For these reasons, we proposed in our original grant to also measure the level of resorptive and formative markers of bone turnover. These measures give us important early insight into the effectiveness of the dietary interventions and assist us in the interpretation of the change in bone indicated by the bone scans. Dr. Caren Gundberg of Yale University Medical School is one of the original collaborators named on this grant. In the proposal, she agreed to perform serum total and undercarboxylated osteocalcin, bone alkaline phosphatase (markers of bone formation) and urine N-telopeptide of collagen type I of bone (NTx) (marker of bone resorption) assays on samples taken at baseline and at 6 month intervals for two years. In addition to information on the bone response to these dietary changes, we can also gain insight into the bioavailability of vitamin K_1 from the measure of osteocalcin and undercarboxylated osteocalcin. Dr. Gundberg is collaborating with the Principal Investigator, Dr. Calvo, on another grant sponsored by FDA's Office of Women's Health where they are measuring both intact, and fragmented osteocalcin and undercarboxylated osteocalcin.

Scope of Work:

The Project Officer/Principal Investigator will provide the contractor with approximately 160 serum and 160 "spot" urine samples obtained five (5) times over the course of the study at baseline and at six (6) month intervals over two (2) years for a total of approximately 800 serum and 800 urine samples. The contractor, Dr. Gundberg, shall analyze each set of serum samples for total intact osteocalcin, undercarboxylated osteocalcin, and bone specific alkaline phosphatase using the same methods as she used in the NHANES III analyses. The contractor shall analyze each set of "spot" urine collections for the level of the resorptive marker of bone turnover, N-terminal cross-linking peptide of type one collagen of bone (NTx), expressed in molar equivalents of bone type I collagen and normalized to creatinine content using the assay kit manufactured by Ostex International (Seattle, WA). Alternatively, the contractor may elect to measure this bone resorptive marker (NTx) in serum if the assay is available at the same lower price as the urine assay. The serum assay has the advantage of greater stability and is not subject to the confounding effect of variation in renal clearance and creatinine determination. The serum NTx assay has also recently received FDA approval. Quality control must be routinely monitored by assaying three unknown serum

samples (low, medium and high) provided by Yale as internal controls and one serum pool provided by CFSAN/FDA. Assays must strictly adhere to acceptable limits of error established for each of the controls (mean $\pm$ 2 SD). All resulting data must be reviewed prior to reporting any results in order to establish that all point fall within the acceptable range. If a source of error is discovered, the assay must be re-established and the samples repeated. To assure that no assay drift occurs, three random samples should be repeated from the previous assay batch. Westgard rules apply to all quality control monitoring and all quality control data are maintained in chart form.

Deliverables:

The contractor shall provide within six months of receiving each of five sets of sera (160 samples in each set) from the Project Officer/ Principal Investigator at FDA:

(1) A written report presenting the serum levels of intact osteocalcin, undercarboxylated osteocalcin and bone specific alkaline phosphatase for each subject sample in that sera set and level of NTx expressed in molar equivalents of type I collagen of bone corrected for creatinine content for each set of urine samples. In addition, the contractors will report specific information as to the procedure used to perform each sera assay and assay performance characteristics including inter- and intra-assay coefficients of variation for both the contractors' internal controls and serum controls (pools) provided by CFSAN/FDA .

(2) A final report is due within six months of receipt of the final set of sera and urine samples which summarizes the results of all the analyses and plots the assay performances over the duration of the study. The contractor shall comment on the overall quality of the assay performance for each of the four (4) analytes measured and present evidence of quality control measures that were observed over the study duration.

Period of Performance: July 31, 2000 to October 31, 2002

4. **Independent Government Cost Estimate (IGCE):**

$49,280

5. **Statutory Authority Permitting Other than Full and Open Competition:**

41 U.S.C. 253(c)(1)

6. **Demonstration that the Contractor's Qualifications of Nature of the Acquisition Required Use of the Cited Authority:**

Dr. Gundberg is an internationally recognized expert on the development and use of bone markers, especially osteocalcin, and its alternative use as an indicator of vitamin K status. Dr. Gundberg earned a Ph.D. in Biochemistry from Boston University and she has extensive postdoctoral experience in Clinical Chemistry from Harvard Medical School. She has published well over 50 papers in prestigious peer-reviewed journals and numerous chapters and reports largely dealing with aspects of the vitamin K-dependent proteins, osteocalcin and others. Evidence of her wide recognition as an expert in this field, includes a recent report she finished that was sponsored by the National Osteoporosis Foundation to address some controversial aspects of the clinical application of and interpretation of markers of bone turnover. Dr Gundberg and Dr. Calvo published a very lengthy review of the molecular basis and clinical applications of biological markers of bone turnover in *Endocrine Reviews* in 1996.

7. **Description of Efforts Made to Ensure that Offererors are Solicited from as Many Potential Sources as is Practicable:**

No efforts have been made. Dr. Gundberg is the sole source of the specific antibodies used in the analyses of all the osteocalcin components in past joint studies with FDA. It is imperative that we use the same antibodies developed by Dr. Gundberg to measure intact and undercarboxylated osteocalcin, because different antibodies, although specific for osteocalcin, have different binding characteristics and recognize different areas of the osteocalcin molecule, some of which may be fragments rather than the intact osteocalcin molecule. Use of any other immunoassay system would only confound interpretation and needed extrapolation of the data. Sole use of her assays will also allow consistency of data from several other projects that Dr. Gundberg has collaborated on with FDA and NCHS in the measurement of: 1) osteocalcin in more than 3,000 race/age diverse surplus serum samples from NHANES III Survey; 2) intact and fragmented and undercarboxylated osteocalcin 4,000 samples from men and women 20 years of age and older from NHANES III; and 3) intact and undercarboxylated osteocalcin as a functional indicator of vitamin K status in sera from girls and young adult women . Use of any other commercial or non-commercial assay for osteocalcin or undercarboxylated osteocalcin would preclude comparison between subjects from the present project and those subjects from the NHANES III survey, which is nationally representative. In addition, Dr. Gundberg can offer the assay of the urine resorption marker at considerable savings as the company has offered to discount it to her. The NTx marker is produced by only one company which owns the patent on it, and it was the resorptive marker chosen to be measured in future NHANES Surveys and those currently underway. The P-I does not have the needed equipment to run this assay at the FDA and for this reason asked Dr. Gundberg to perform them.

8. **Determination by the Contracting Officer that the Anticipated Cost will be Fair and Reasonable:**

Page 25
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Dr. Gundberg has agreed to run the samples at an individual cost of $56.00 per sample based on a total delivery of approximately 800 samples. This is a very fair price considering that it covers the measurement of osteocalcin, undercarboxylated osteocalcin, and bone specific alkaline phosphatase levels measured in duplicate in each serum sample and NTx measured in duplicate and expressed per gram of creatinine in "spot" urine samples. This price includes materials, labor, standards, controls, and repeat analyses. Hospital Clinical Chemistry units charge range from $45.00 to $90.00 per individual sample for NTx measurement alone. Yale University charges an indirect fee on all service analyses of 10% on all analytical samples that must be added onto the total cost. The total cost of these analyses that will be conducted over the next two years is **($61.60 per sample X 800 urine and serum samples)** is **$49,280.**

9. **Description of the Market Survey Conducted or Statement of the Reasons Why Survey was not Conducted:**

Dr. Gundberg has participated in, organized and chaired several satellite symposia at the annual meetings of the American Society for Bone and Mineral Research addressing the clinical application and interpretation of the use of bone markers and in the use of undercarboxylated osteocalcin as a functional indicator of vitamin K nutritional status. In addition, she has over ten years of collaborative research experience with the Project Officer / Principal Investigator and is a named collaborator on this grant.

10. **Other Facts Supporting the Use of Other Than Full and Open Competition:**

Not applicable

SIGNATURE PAGE

I certify that this requirement is subject to annual review by senior management and, as a result of that review, this action is approved based a current need and cost benefit to the program mission.

07/05/00

Project Officer Date

APPROVED:

7/5/00

Director, Office of Nutritional Products, Labeling and Dietary Supplements Date

7/7/00

for Leader, Budget Execution and Fiscal Team Date

7/7/00

Director, Division of Planning and Financial Resources Management Date

7/7/00

for Director, Office of Management Systems Date

DEPARTMENT OF
HEALTH AND HUMAN SERVICES

PURCHASE/SERVICE/STOCK REQUISITION

REQUISITION NUMBER: X-24831

OFFICE CODE/SYMBOL:

BPA and Call No. _____

D Division of Contracts and Procurement Mgmt. OFACS	REQUEST FOR ☐ PURCHASE ☒ SERVICE ☐ STOCK ISSUE ☐ RENTAL/LEASE		
EQUESTING ORGANIZATION Food and Drug Administration	CUSTODIAL AREA CFSAN	DATE 07-10-2000	OBJECT CLASS 25.55
OR REFERENCE CALL Juanita Pointer	EXTENSION 205-4098	APPROPRIATION 7500600 22390D/10	
ELIVER TO Mona S. Calvo, Ph.D. (HFS-452) Office of Special Nutritionals, CFSAN 200 C. Street, SW Wash., DC 20204		CAN 0-6991697-X-24831 DATE REQUIRED	

ITEM NO.	DESCRIPTION (INCLUDE STOCK NUMBER, MODEL/PART NO., ETC.)	QUANTITY REQUIRED	UNIT OF ISSUE	COST UNIT	COST TOTAL
	Measurement of Serum IGF-1 as an Indicator of the Efficacy of Dietary Intervention Strategies to Promote Bone Mass Acquistion in U.S. Naval Academy Midshipmen Period of Performance: July 31, 2000 July 31, 2002 Source: Clifford J. Rosen, MD Chief of Medicine St. Joseph's Hospital Reserach Professor of Nutrition University of Maine Maine Center for Osteoporosis Research and Education 360 Broadway Bangor, Maine 04401 Phone 207-262-1920			27,300.00	27,300.00

certify that the property/services requested are required or Government business, and are not available from excess or current assets.*

FUNDS AVAILABLE (Signature/Title): Barbara Gautreaux, Leader, BEFPT — DATE: 7/12/00

TOTAL: $27,300.00

REQUESTED BY (Signature/Title)* Mona S. Calvo, Ph.D., Project Officer	DATE 7-10-2000	RECEIVING OFFICIAL - I certify that the quantities indicated in the "Quantity Required" column above have been received in total or as annotated.	
RECOMMEND APPROVAL (Signature/Title)* Lori Love, MD., Ph.D., Chief, CRRS, ONPLDS Director	DATE 07/10/2000	RECEIVING OFFICIAL (Signature/Title)*	DATE
APPROVED BY (Signature/Title)* Christine J. Lewis, Ph.D., Act Dir ONPLDS	DATE 7/10/2000	ORDER NO. (PO, DO, FEDSTRIP, ETC.)	ORDER DATE
PROPERTY MANAGEMENT OFFICER (Signature)*	DATE	VOUCHER NO.	

HHS-393 (Rev. 6/90)

This form was electronically produced by Elite Federal Forms, Inc.

Requisition Number: X-24831

Justification for Other than Full and Open Competition

1. **Identification of the Agency and the Contracting Activity**

Agency : Food and Drug Administration
PHS/DHSS

Program Officer: Mona S. Calvo, Ph.D. HFS-805

Contracting Activity: Division of Contracts and Procurement Management HFA-512

2. **Nature of Action Being Approved:**

Full and open competition is restricted. The acquisition must be from a specified source:

Clifford J. Rosen, MD
Chief of Medicine
St. Joseph's Hospital
Research Professor of Nutrition
University of Maine,
Maine Center for Osteoporosis Research and Education
360 Broadway, Bangor, Maine 04401
Phone: 207-262-1920

3. **Description of Supplies or Services and Estimated Value:**

Title: Measurement of Serum IGF-I as an Indicator of the Efficacy of Dietary Intervention Strategies to Promote Bone Mass Acquistion in U.S. Naval Academy Midshipmen.

Background:

In response to a proposal submitted to meet the objective of a Request for Proposals focusing on strategies to improve bone health in military men and women, CFSAN was awarded funding from the Department of Defense to conduct a two-year dietary intervention study (IAG No. 224-98-2568). This study involves supplementing male and female Midshipmen from the United States Naval Academy with calcium and specific nutrients which have been identified as limited in the diet and essential to bone matrix growth and mineralization. The dietary supplements are intended to optimize all nutritional factors to allow maximum gain in bone mass or density in this last phase of bone growth. Because denser bones are stronger, the risk of osteoporosis and bone fracture in old age is in part

determined by peak bone mass or the total amount of bone mass achieved at skeletal maturity. We know that calcium is important to gain in bone mass in young children, but we do not know its significance in young adults whose bones have achieved full linear growth.

The goal of this study is to determine if optimizing the nutrient intake of all the micro-nutrients needed for bone growth, not just calcium, will effectively increase gain in bone mass in young adults as it does in growing children. One mechanism through which essential nutrients other than calcium, such as zinc and protein, may modulate gain in peak bone mass is through the stimulation of insulin-like growth factor (IGF-1). IGF-1 is a peptide hormone that has been shown to have a positive association with bone mass in young adults and elderly patients with hip fractures. The activity of IGF-1 is regulated or limited by higher levels of a specific type of binding protein, insulin-like growth factor binding protein-4 (IGFBP-4) which in turn is modulated by parathyroid hormone, the hormone that regulates the resorption of bone. There is a complex hormonal interaction between these factors and all of these can be modified by the diet. We need to measure IGF-1 and IGFBP-4 in this study to help us determine what mechanisms are operative with theses dietary interventions, particularly the supplement regimen that effectively promotes gain in bone.

Dr. Clifford Rosen, Chief of Medicine at St. Joseph Hospital and Research Professor of Nutrition in Maine's Center for Osteoporosis Research and Education at the University of Maine is one of the original collaborators named on this grant. Dr. Rosen's contributions to this study are in the measurement of serum Insulin-like Growth Factor –1 and IGFBP-4. All other hormones that provide insight into the mechanisms operative in dietary induced increase in bone mass will be measured by the Principal Investigator at the Mod-1 laboratories.

Scope of Work:

The Project Officer/Principal Investigator will provide the contractor with serum samples obtained five (5) times over the course of the study at baseline and at six (6) month intervals over two (2) years for a total of approximately 700 serum samples. The contractor shall analyze each set of serum samples for IGF-1 and total IGFBP-4 levels. Both measures utilize radioimmunoassays (RIA) established in Dr. Rosen's laboratory at St. Joseph's Hospital. Measurement of IGF-1 involves RIA following ethanol extraction of serum, while measurement of IGFBP-1 uses a specific RIA on un-extracted serum. Assays must strictly adhere to acceptable limits of error established for each of the controls (mean $\pm$ 2 SD). All resulting data should be reviewed prior to reporting any results in order to establish that all points fall within the acceptable range. If a significant source of error is discovered, the assay must be re-established and the samples repeated. To assure that no assay drift occurs, three random samples should be repeated from the previous assay batch. Westgard rules apply to all quality control monitoring and all quality control data are maintained in chart form.

Deliverables:

The contractor shall provide within six months of receiving each of five sets of sera (approximately 160 samples in the first set and 135 samples in the next 4 sets) from the Project Officer/ Principal Investigator at FDA:

(1) A written report presenting the serum levels of IGF-1 and IGFBP-4 in each sample for each of the five separate sets of sera. In addition, the contractors will report specific information as to the procedure used to perform each sera assay and assay performance characteristics including inter- and intra-assay coefficients of variation for both the contractors' internal controls and serum controls (baseline serum pools) provided by CFSAN/FDA.

(2) A final report is due within six months of receipt of the final set of sera and urine samples which summarizes the results of all the analyses and plots the assay performances over the duration of the study. The contractor shall comment on the overall quality of the assay performance for IGF-1 and IGFBP-4 by presenting evidence of quality control measures that were observed over the study duration.

Period of Performance: July 31, 2000 to July 31, 2001

4. **Independent Government Cost Estimate (IGCE):**

$27,300

5. **Statutory Authority Permitting Other than Full and Open Competition:**

41 U.S.C. 253(c)(1)

6. **Demonstration that the Contractor's Qualifications of Nature of the Acquisition Required Use of the Cited Authority:**

Dr. Rosen is an internationally recognized endocrinologist with expertise in the area of osteoporosis. Among his varied research activities, he is well-known for his work on dietary factors such as zinc that influence the secretion of the hormone Insulin-like Growth Factor-1 that has been shown to stimulate increase in bone mass. Dr. Rosen was asked to collaborate on this research project by providing the analyses of this hormone which should increase in response to the specially designed protein dietary supplement fed in the study.

7. **Description of Efforts Made to Ensure that Offererors are Solicited from as Many Potential Sources as is Practicable:**

No efforts have been made. Dr. Rosen's laboratory is the only source that I am aware of where a clinical research laboratory measures both IGF-1 and IGFBP-4 for collaborating researchers and provides needed expertise on the interpretation of the results as they relate to specific nutrient intake. He was specifically asked to collaborate with CFSAN on this grant by providing these analyses. Dr, Rosen's unique expertise in endocrinology and nutrition is vital to interpreting the changes in serum levels of this important growth regulating hormone and its binding protein and how they relate to accretion of bone mass with specific dietary supplement regimens.

8. **Determination by the Contracting Officer that the Anticipated Cost will be Fair and Reasonable:**

Dr. Rosen has agreed to run the samples at an individual cost of $ 39.00 per sample based on a total delivery of approximately 700 samples. This is a very fair price considering that it covers the measurement of IGF-1 at $15.00 and IGFBP-4 at $24 per sample. IGF-1 and IGFBP-4 levels will be measured in duplicate in each serum sample. This price includes materials, labor, standards, controls, and repeat analyses. Hospital Clinical Chemistry units do not routinely measure IGF-1 or its binding protein since they are usually associated with clinical research not diagnostic procedures. The total cost of these analyses that will be conducted over the next two years is ($39.00 per sample X 700 serum samples) is $27,300.

9. **Description of the Market Survey Conducted or Statement of the Reasons Why Survey was not Conducted:**

Dr. Rosen has participated in, organized, and chaired several national and international symposia on endocrine and dietary factors involved in osteoporosis and other disorders of bone. He is presently on the Editorial Boards of Calcified Tissues International, and Journal of Bone and Mineral Research, and Editor-in Chief of the Journal of Clinical Densitometry. He has authored over 80 peer-reviewed papers and holds the positions of Chief of Medicine, St. Joseph Hospital, Maine Center for Osteoporosis Research and Education and he is a Research Professor of Nutrition at the University of Maine. Dr. Rosen is a world recognized authority on the role of serum IGF-1 on bone accretion and the influence of physical activity and specific dietary factors such as zinc on serum levels of this growth hormone. Dr. Rosen's expertise and knowledge of the complexity of IGF-1's regulation by diet and other hormones is the key factor in our seeking his collaboration on this project. He is unique in that he can measure both IGF-1 and its binding protein and based on his previous research experience, he will help to accurately interpret the mechanisms through which bone acquisition is stimulated by dietary intervention.

DEPARTMENT OF HEALTH AND HUMAN SERVICES

PURCHASE/SERVICE/STOCK REQUISITION

REQUISITION NUMBER: X-24822

OFFICE CODE/SYMBOL:

BPA and Call No. _____

TO: Division of Contracts and Procurement Mgmt. OFACS	REQUEST FOR: ☐ PURCHASE ☒ SERVICE ☐ STOCK ISSUE ☐ RENTAL/LEASE		
REQUESTING ORGANIZATION: Food and Drug Administration	CUSTODIAL AREA: CFSAN	DATE: 11-9-99	OBJECT CLASS: 25.38
FOR REFERENCE CALL: Juanita Pointer	EXTENSION: 205-4098	APPROPRIATION: 7500600 22390D/10	
DELIVER TO: Mona S. Calvo, Ph..D. (HFS-452) Office of Special Nutritionals, CFSAN 200 C. Street, SW Wash., DC 20204		CAN: 0-6991697-X-24822 DATE REQUIRED:	

ITEM NO.	DESCRIPTION (INCLUDE STOCK NUMBER, MODEL/PART NO., ETC.)	QUANTITY REQUIRED	UNIT OF ISSUE	COST UNIT	COST TOTAL
	Memorandum of Need - New Interagency Agreement IAG Number: 224-00-2600 Title: Bone Mineral Density Determinations (A Dietary Strategy to Maximize Bone Mass in United States Naval Academy Midshipmen) Period of Performance and Amount Available of Obligation 12 Months			28,300.00	28,300.00
	Director, Office of Financial Management				

I certify that the property/services requested are required for Government business, and are not available from excess or current assets.*

FUNDS AVAILABLE (Signature/Title): for Carl Chrisman for Darryl Patterson, Act. Ldr. BEFPT

DATE: 12/22/99

TOTAL: $28,300.00

REQUESTED BY (Signature/Title)*: for Mona S. Calvo, Ph.D. Project Officer — DATE: 12/16/99

RECOMMEND APPROVAL (Signature/Title)*: for Lori Love, MD., Ph.D., Chief, CRRS, OSN — DATE: 12/20/99

APPROVED BY (Signature/Title)*: for Elizabeth Yetley, Ph. D., Director, OSN — DATE: 12/23/99

PROPERTY MANAGEMENT OFFICER (Signature)* — DATE

RECEIVING OFFICIAL - I certify that the quantities indicated in the "Quantity Required" column above have been received in total or as annotated.

RECEIVING OFFICIAL (Signature/Title)* — DATE

ORDER NO. (PO, DO, FEDSTRIP, ETC.) — ORDER DATE

VOUCHER NO. — VOUCHER DATE

HHS-393 (Rev. 6/90)

SIGNATURE PAGE

PROJECT TITLE: Bone Mineral Density Determinations (A Dietary Strategy to Maximize Bone Mass in United States Naval Academy Midshipmen)

IAG NUMBER: 224-00-2600

FUNDING DATA: See Attached HFS-393 for (FY-00 Funding)

Project Officer

APPROVED:

Chief, Clinical Research and Review Staff — Date 12/21/99

Director, Office of Special Nutritionals — Date 12/20/99

Acting Team Leader, Budget Execution and Fiscal Policy Team — Date 12/22/99

Director, Division of Planning and Financial Resources Management — Date 12/22/99

Director, Office of Management Systems — Date 12/28/99

Director, Center for Food Safety and Applied Nutrition — Date 12/28/99

Director, Office of Financial Management — Date

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration

INTERAGENCY AGREEMENT

1. IAG NO. (FDA)	2. TYPE OF AGREEMENT	3. MODIFICATION NO.
224-90-2600	☒ New ☐ Mod ☐ Administrative	

4. TITLE OF PROJECT

Bone Mineral Density Determinations (A Dietary Startegy to Maximize Bone Mass in United State Naval Academy Midshipmen)

5. DESCRIPTION OF WORK - ATTACHED	6. AMOUNT	
Attached	$28,300.00	
7. NAME AND ADDRESS OF PARTICIPATING FEDERAL AGENCY Office of Naval Research (Code 341) 800 North Quincy Street Arlington, VA 22217	LIAISON NAME: Dr. John Thomas	PHONE NO. (703) 696-0369 FAX NO. ( )
8. NAME AND ADDRESS OF PARTICIPATING FDA UNIT CFSAN/FDA 200 C. Street, SW Wash., DC 20204	LIAISON NAME: Mona Calvo, Ph.D.	PHONE NO. (202) 205-4198 FAX NO. ( )

9. PERIOD OF AGREEMENT FROM: 10-01-1999 THROUGH: 09-30-2000

This agreement may be terminated by either party upon a thirty day advance written notice.

10. AUTHORITY (FDA)

- ☐ Economy Act approved June 30, 1932, as amended by 31 U.S.C. 1535
- ☒ Section 301 of the Public Health Service Act (42 USC 241)
- ☐ Other (specify) _____

11. AUTHORITY *(Other Agency)*

12. FDA FUNDING INFORMATION

- ☒ Increase from 0 by 28,300 to 28,300
- ☐ Decrease from _____ by _____ to _____

CAN: 0-6991697-X-24822
Obj. Class: 25.38
PMS Codes: 7500600 22390D/I0

Administrative billing requirements will comply with GAO Policy and Procedures, Title 7, Section 8.4

- ☐ Billing: _____ OPAC system FDA ALC 75060099 Other Agency ALC _____
- ☒ _____ SF 1080 - FDA (HFA-120) 5600 Fishers Lane, Rockville, MD 20857

13. PARTICIPATING AGENCY FUNDING INFORMATION

a. Legal authority for the acquisition of supplies/services exists within your agency.
b. This action does not conflict with any other agency's authority or responsibility.

14. PARTICIPATING AGENCY IS

- ☐ Required to sign
- ☒ Not required to sign

15. FDA ACCEPTANCE	16. PARTICIPATING AGENCY ACCEPTANCE
SIGNED: _____	SIGNED: _____
NAME: Rosemary Springer	NAME: _____
TITLE: Senior Grants Management Specialist, OFACS	TITLE: _____
DATE: 12-13-1999	DATE: 12-13-1999

FDA 3443 (7/96)

Project Title : Bone Mineral Density Determinations for Joint FDA Naval Academy Project Entitled, *A Dietary Strategy to Maximize Bone Mass in United States Naval Academy Midshipmen.*

Scope of Work:

The amount of $28,300 is awarded by the U.S. Food and Drug Administration to the Office of Navy Research for work performed toward the conduct of the joint FDA-Naval Academy project entitled *A Dietary Strategy to Maximize Bone Mass in the United States Naval Academy Midshipmen.* The work to be accomplished in all (approximately) 320 Naval Academy Midshipmen Class of 2003 participating in the study will occur over no more than 3 years as described in the original proposal on page 21. It consists of use of the dual energy-X-ray absorbtiometer (DEXA,Norland XR-36) measurement (at baseline and years one and two) of bone mineral density (BMD) and bone mineral content (BMC) for the total body and at five skeletal sites including the femoral neck, trochanter, Ward's triangle, lumbar spine, and distal tibia. The fixed price of $30 per scan includes anthropometric measures conducted at the time of each DEXA scan by a trained and experienced technician for each subject participating in the study . These measurements include height using a calibrated stadiometer, weight using a calibrated electronic scale, body composition by skin fold using Lange caliper and body girth measurements using a precise tape measure. The technician will also administer a simple pregnancy test (urine indicator) immediately prior to each bone scan in all participating females Midshipmen. Results of these tests will be maintained in strict confidence, but no DEXA scans will be performed in women with a positive pregnancy test. These determinations will be supervised by the Co-Principal Investigator, Dr. David Armstrong, of Bethesda Naval Hospital and the medical monitoring of this task will be carried out by CAPT. Robert Schultz, USN MC, of the Naval Clinic at Annapolis, MD. All collected data measures will be reported directly and electronically only to the study biostatistician, Dr. Curtis N. Barton, located at 200 C St. S.W., Washington, DC in order to maintain full and complete privacy for each subject participating in the study. Annual reports demonstrating quality control monitoring of the DEXA and ~~ultrasound~~ anthropometric measures will be made to the Principal Investigators listed above. No other deliverables or reports are anticipated. Local travel expenses to cover travel to and from the U.S. Naval Academy for the Principal Investigators and costs of miscellaneous supplies are covered in this award.

Unpublished Data
Baseline Bone Density and Bone Mineral Content Data

1. Descriptive Characteristics of the Baseline Gender and Treatment groups.
A. Age in Months: Mean values for each gender.

SEX	N	MEAN	STD	STDERR
F	52	233.02	10.20	1.41
M	109	239.77	14.50	1.39

B. Age in Months: Mean values for each sex and treatment group.

SEX	TREATMNT	N	MEAN	STD	STDERR
F	CORAL SEA	14	235.07	15.00	4.01
F	LEXINGTON	13	229.15	8.31	2.30
F	MIDWAY	14	235.86	5.56	1.49
F	YORKTOWN	11	231.36	8.65	2.61
M	CORAL SEA	27	240.89	18.26	3.51
M	LEXINGTON	25	239.48	10.84	2.17
M	MIDWAY	29	239.21	13.24	2.46
M	YORKTOWN	28	239.54	15.23	2.88

C. Body Mass Index: Mean values for each gender.

SEX	N	MEAN	STD	STDERR
F	52	23.00	2.08	0.29
M	109	24.40	2.26	0.22

D. Body Mass Index: Mean values for each sex and treatment group.

SEX	TREATMNT	N	MEAN	STD	STDERR
F	CORAL SEA	14	23.30	2.30	0.61
F	LEXINGTON	13	23.25	2.15	0.60
F	MIDWAY	14	22.62	1.89	0.51
F	YORKTOWN	11	22.78	2.14	0.64
M	CORAL SEA	27	24.27	2.15	0.41
M	LEXINGTON	25	24.33	2.22	0.44
M	MIDWAY	29	24.45	2.40	0.45
M	YORKTOWN	28	24.51	2.36	0.45

Unpublished Data
Baseline Bone Density and Bone Mineral Content

E. Bone Mineral Content (gm) measured at six skeletal sites (distal tibia, femoral neck, total body, trochanter, lumbar vertebra (L2, L3,L4) and Ward's triangle). Mean values for each gender.

SEX	SITE	N	MEAN	STD	STDERR
F	TIBIA	52	6.47	0.78	0.11
F	fem neck	52	4.67	0.95	0.13
F	total	52	2761.13	361.77	50.17
F	troch	52	10.32	2.21	0.31
F	vert L2	52	14.70	2.79	0.39
F	vert L3	52	16.38	2.75	0.38
F	vert L4	52	16.72	2.90	0.40
F	wards tri	52	0.91	0.13	0.02
M	TIBIA	109	7.83	0.83	0.08
M	fem neck	109	6.27	0.81	0.08
M	total	108	3373.85	356.35	34.29
M	troch	109	15.33	2.80	0.27
M	vert L2	109	18.52	3.18	0.30
M	vert L3	109	21.10	3.85	0.37
M	vert L4	109	20.96	3.97	0.38
M	wards tri	109	0.95	0.13	0.01

F. Bone Mineral Density (gm/cm^2) measured at six skeletal sites (distal tibia,femoral neck, total body, trochanter, lumbar vertebra (L2,L3,L4) and Ward's triangle). Mean values for each gender.

SEX	SITE	N	MEAN	STD	STDERR
F	TIBIA	52	1.389	0.124	0.017
F	fem neck	52	1.060	0.122	0.017
F	total	52	1.001	0.085	0.012
F	troch	52	0.837	0.108	0.015
F	vert L2	52	1.103	0.142	0.020
F	vert L3	52	1.117	0.136	0.019
F	vert L4	52	1.082	0.125	0.017
F	wards tri	52	0.912	0.130	0.018
M	TIBIA	109	1.568	0.130	0.012
M	fem neck	109	1.159	0.134	0.013
M	total	108	1.099	0.088	0.008
M	troch	109	0.952	0.120	0.011
M	vert L2	109	1.185	0.148	0.014
M	vert L3	109	1.208	0.156	0.015
M	vert L4	109	1.141	0.147	0.014
M	wards tri	109	0.947	0.134	0.013

Unpublished Data
Baseline Bone Density and Bone Mineral Content

2. Bone Mineral Content (gm) measured at six skeletal sites (distal tibia, femoral neck, total body, trochanter, lumbar vertebra (L2, L3,L4) and Ward's triangle). Mean values for gender and treatment group.

A. Female Midshipmen:

SEX	SITE	TREATMNT	N	MEAN	STD	STDERR
F	TIBIA	CORAL SEA	14	6.58	0.86	0.23
F	TIBIA	LEXINGTON	13	6.63	0.74	0.21
F	TIBIA	MIDWAY	14	6.24	0.71	0.19
F	TIBIA	YORKTOWN	11	6.41	0.82	0.25
F	fem neck	CORAL SEA	14	4.53	0.80	0.21
F	fem neck	LEXINGTON	13	5.00	1.02	0.28
F	fem neck	MIDWAY	14	4.76	0.75	0.20
F	fem neck	YORKTOWN	11	4.32	1.20	0.36
F	total	CORAL SEA	14	2667.38	315.66	84.36
F	total	LEXINGTON	13	2802.94	425.01	117.88
F	total	MIDWAY	14	2735.64	200.29	53.53
F	total	YORKTOWN	11	2863.49	491.98	148.34
F	troch	CORAL SEA	14	10.13	1.98	0.53
F	troch	LEXINGTON	13	10.27	2.75	0.76
F	troch	MIDWAY	14	10.31	1.73	0.46
F	troch	YORKTOWN	11	10.64	2.60	0.79
F	vert L2	CORAL SEA	14	14.67	2.70	0.72
F	vert L2	LEXINGTON	13	14.14	3.40	0.94
F	vert L2	MIDWAY	14	14.44	1.59	0.42
F	vert L2	YORKTOWN	11	15.71	3.38	1.02
F	vert L3	CORAL SEA	14	16.23	2.86	0.76
F	vert L3	LEXINGTON	13	15.76	2.87	0.80
F	vert L3	MIDWAY	14	16.04	2.16	0.58
F	vert L3	YORKTOWN	11	17.75	3.01	0.91
F	vert L4	CORAL SEA	14	16.76	2.81	0.75
F	vert L4	LEXINGTON	13	16.00	3.13	0.87
F	vert L4	MIDWAY	14	16.20	2.33	0.62
F	vert L4	YORKTOWN	11	18.19	3.22	0.97
F	wards tri	CORAL SEA	14	0.86	0.11	0.03
F	wards tri	LEXINGTON	13	0.90	0.19	0.05
F	wards tri	MIDWAY	14	0.93	0.10	0.03
F	wards tri	YORKTOWN	11	0.97	0.09	0.03

Unpublished Data
Baseline Bone Density and Bone Mineral Content

B. Male Midshipmen:

SEX	SITE	TREATMNT	N	MEAN	STD	STDERR
M	TIBIA	CORAL SEA	27	7.65	0.76	0.15
M	TIBIA	LEXINGTON	25	8.03	0.78	0.16
M	TIBIA	MIDWAY	29	7.98	0.85	0.16
M	TIBIA	YORKTOWN	28	7.66	0.87	0.16
M	fem neck	CORAL SEA	27	6.21	0.84	0.16
M	fem neck	LEXINGTON	25	6.38	0.66	0.13
M	fem neck	MIDWAY	29	6.28	0.80	0.15
M	fem neck	YORKTOWN	28	6.22	0.92	0.17
M	total	CORAL SEA	26	3385.22	381.46	74.81
M	total	LEXINGTON	25	3386.61	307.72	61.54
M	total	MIDWAY	29	3384.87	381.05	70.76
M	total	YORKTOWN	28	3340.49	363.58	68.71
M	troch	CORAL SEA	27	15.20	2.36	0.45
M	troch	LEXINGTON	25	15.15	2.48	0.50
M	troch	MIDWAY	29	15.72	3.35	0.62
M	troch	YORKTOWN	28	15.21	2.92	0.55
M	vert L2	CORAL SEA	27	18.52	2.63	0.51
M	vert L2	LEXINGTON	25	18.75	3.33	0.67
M	vert L2	MIDWAY	29	18.61	4.20	0.78
M	vert L2	YORKTOWN	28	18.22	2.31	0.44
M	vert L3	CORAL SEA	27	21.05	3.28	0.63
M	vert L3	LEXINGTON	25	21.48	4.05	0.81
M	vert L3	MIDWAY	29	21.27	5.05	0.94
M	vert L3	YORKTOWN	28	20.66	2.73	0.52
M	vert L4	CORAL SEA	27	20.85	3.12	0.60
M	vert L4	LEXINGTON	25	21.42	4.16	0.83
M	vert L4	MIDWAY	29	21.14	5.13	0.95
M	vert L4	YORKTOWN	28	20.45	3.21	0.61
M	wards tri	CORAL SEA	27	0.94	0.15	0.03
M	wards tri	LEXINGTON	25	0.95	0.11	0.02
M	wards tri	MIDWAY	29	0.97	0.13	0.02
M	wards tri	YORKTOWN	28	0.92	0.14	0.03

Unpublished Data
Baseline Bone Density and Bone Mineral Content

3. Bone Mineral Density (gm/cm^2) measured at six skeletal sites (distal tibia, femoral neck, total body, trochanter, lumbar vertebra (L2, L3, L4) and Ward's triangle). Mean values for gender and treatment group.

A. Female Midshipmen:

SEX	SITE	TREATMNT	N	MEAN	STD	STDERR
F	TIBIA	CORAL SEA	14	1.408	0.120	0.032
F	TIBIA	LEXINGTON	13	1.391	0.130	0.036
F	TIBIA	MIDWAY	14	1.364	0.112	0.030
F	TIBIA	YORKTOWN	11	1.394	0.146	0.044
F	fem neck	CORAL SEA	14	1.015	0.106	0.028
F	fem neck	LEXINGTON	13	1.047	0.164	0.045
F	fem neck	MIDWAY	14	1.072	0.082	0.022
F	fem neck	YORKTOWN	11	1.118	0.114	0.034
F	total	CORAL SEA	14	0.989	0.084	0.023
F	total	LEXINGTON	13	0.987	0.092	0.026
F	total	MIDWAY	14	0.998	0.061	0.016
F	total	YORKTOWN	11	1.036	0.105	0.032
F	troch	CORAL SEA	14	0.825	0.114	0.030
F	troch	LEXINGTON	13	0.818	0.133	0.037
F	troch	MIDWAY	14	0.839	0.094	0.025
F	troch	YORKTOWN	11	0.871	0.089	0.027
F	vert L2	CORAL SEA	14	1.110	0.136	0.036
F	vert L2	LEXINGTON	13	1.027	0.151	0.042
F	vert L2	MIDWAY	14	1.085	0.091	0.024
F	vert L2	YORKTOWN	11	1.208	0.143	0.043
F	vert L3	CORAL SEA	14	1.105	0.140	0.037
F	vert L3	LEXINGTON	13	1.056	0.136	0.038
F	vert L3	MIDWAY	14	1.099	0.087	0.023
F	vert L3	YORKTOWN	11	1.225	0.136	0.041
F	vert L4	CORAL SEA	14	1.064	0.120	0.032
F	vert L4	LEXINGTON	13	1.032	0.130	0.036
F	vert L4	MIDWAY	14	1.069	0.078	0.021
F	vert L4	YORKTOWN	11	1.182	0.134	0.040

Unpublished Data
Baseline Bone Density and Bone Mineral Content

A. Female Midshipmen (continued):

SEX	SITE	TREATMNT	N	MEAN	STD	STDERR
F	wards tri	CORAL SEA	14	0.858	0.107	0.029
F	wards tri	LEXINGTON	13	0.901	0.185	0.051
F	wards tri	MIDWAY	14	0.932	0.103	0.028
F	wards tri	YORKTOWN	11	0.966	0.094	0.028

B. Male Midshipmen:

SEX	SITE	TREATMNT	N	MEAN	STD	STDERR
M	TIBIA	CORAL SEA	27	1.552	0.122	0.023
M	TIBIA	LEXINGTON	25	1.593	0.140	0.028
M	TIBIA	MIDWAY	29	1.578	0.136	0.025
M	TIBIA	YORKTOWN	28	1.553	0.127	0.024
M	fem neck	CORAL SEA	27	1.148	0.152	0.029
M	fem neck	LEXINGTON	25	1.173	0.108	0.022
M	fem neck	MIDWAY	29	1.168	0.118	0.022
M	fem neck	YORKTOWN	28	1.147	0.157	0.030
M	total	CORAL SEA	26	1.101	0.095	0.019
M	total	LEXINGTON	25	1.099	0.079	0.016
M	total	MIDWAY	29	1.114	0.080	0.015
M	total	YORKTOWN	28	1.080	0.097	0.018
M	troch	CORAL SEA	27	0.947	0.116	0.022
M	troch	LEXINGTON	25	0.952	0.109	0.022
M	troch	MIDWAY	29	0.983	0.119	0.022
M	troch	YORKTOWN	28	0.924	0.133	0.025
M	vert L2	CORAL SEA	27	1.184	0.160	0.031
M	vert L2	LEXINGTON	25	1.185	0.144	0.029
M	vert L2	MIDWAY	29	1.202	0.172	0.032
M	vert L2	YORKTOWN	28	1.170	0.118	0.022
M	vert L3	CORAL SEA	27	1.206	0.172	0.033
M	vert L3	LEXINGTON	25	1.203	0.156	0.031
M	vert L3	MIDWAY	29	1.223	0.180	0.034
M	vert L3	YORKTOWN	28	1.198	0.116	0.022
M	vert L4	CORAL SEA	27	1.145	0.170	0.033
M	vert L4	LEXINGTON	25	1.127	0.134	0.027
M	vert L4	MIDWAY	29	1.164	0.171	0.032
M	vert L4	YORKTOWN	28	1.126	0.107	0.020

Unpublished Data
Baseline Bone Density and Bone Mineral Content

B. Male Midshipmen (continued):

SEX	SITE	TREATMNT	N	MEAN	STD	STDERR
M	wards tri	CORAL SEA	27	0.940	0.152	0.029
M	wards tri	LEXINGTON	25	0.952	0.107	0.021
M	wards tri	MIDWAY	29	0.974	0.134	0.025
M	wards tri	YORKTOWN	28	0.920	0.140	0.026

UPI # _____ Date:_____

Please circle the beverage(s) you consume most often (including water) and indicate approximately how many times per week you drink and the usual amount or volume you drink most of the time:

Frequency Consumed Per week **Usual Volume Consumed Each Time**

_____MILK (Type of milk: skimmed, whole, low-fat)
Half to One cup on cereal _____ 8 oz glass _____ 16 oz or more_____

_____CARBONATED SODA (Diet Coke, 7-Up, Mountain Dew, etc.)

Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz____

_____SPORT DRINKS (gatorade, poweraid, etc)

Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz_____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz_____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz_____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz ____
Brand name_____ 12 oz cans__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz ____

_____FRUIT JUICE OR FRUIT PUNCHES AND ICED TEAS (Snapple, etc)
Brand name_____ 8- 12 oz __ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz_____
Brand name_____ 8- 12oz__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz_____
Brand name_____ 8-12oz__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz_____
Brand name_____ 8-12oz __ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz ____
Brand name_____ 8-12oz__ 16 oz bottles___ 20 oz ___ 32 oz____ 64 oz ____

_____COFFEE, TEA, HOT CHOCOLATE

Coffee_____ cups/day Tea _____ cups/day
Hot Chocolate _____ cups/day

_____ WATER (Bottled, carbonated, carbonated /flavored, fountain and tap)

Bottled_____ 8oz glasses/day
Carbonated_____ 8 oz glasses/ day
Carbonated/flavored _____ 8 oz glasses/day
Fountain and Tap_____ 8 oz glasses/day

UPI #_____

Date:_____ Page 1 (Retained by the Study Director)

Information for First Food Record

Please complete the following background information for your food record:

1. Are you currently taking any prescription or over the counter medications? (Please include birth control prescriptions in this category) Yes____ No____

   If you answered yes, please note what you are taking, the frequency of use, the amount, and the approximate length of time you have been taking it:

   _____

   _____

   _____

2. Are you currently taking any type of nutritional supplement or ergogenic aid such as the examples shown in the interview room? Yes____ No____

   If you answered yes, please note what you are taking, the dose and frequency, and the approximate time that you have been taking it: _____

   _____

   _____

3. Have you sustained any injuries since attending the Naval Academy? Yes____

   No____

   If you sustained any injury, please note specifically what the injury was and when it occurred. _____

   _____

4. If you are a female Midshipmen, have you experienced a menstrual cycle in the following months (please check if yes)?
   July____August____September____October____
   Please give the approximate date of the last day of your last menses.

   _____

UPI #_____

Date:_____ Page 2

Information Recorded During and After Food Intake Record

1. Please specify your exercise habits on the days you are recording your food intakes:

   Day 1:_____

   _____

   _____

   Day 2:_____

   _____

   _____

   Day 3: _____

   _____

   _____

2. Please indicate at the end of the 3-day food record whether you believe these 3 days are typical of the way you usually eat.

   _____Typical of the quantity and type of food you usually eat during the week and weekend

   _____Not typical for the quantity and type of food you usually eat for at least one day due to stress or illness, or any other reason. Please specify the day that was not typical_____

   _____Not typical for the quantity and type of food you usually eat for two or more days due to any reason. Please specify the days that were not typical_____

UPI #_____

Date:_____

Information and Directions for 24-hour Urine Collection

The best way we have of easily understanding how well you absorb the minerals that you eat such as calcium, sodium, phosphorus, magnesium and potassium, is to examine what you eat in a day as well as what you excrete in the urine. To do this, we need to make sure that we collect all the urine that you excrete over the 24 hours or day that we monitor what you eat. We also need to make sure that all these minerals stay in solution so we place a very strong acid in the urine collection bottle, consequently you must be careful not splash this on yourself when making a collection.

To collect all the urine you produce in a period of 24 hours you must take your urine jug with you during the day. Collections are most difficult at night because people are often half asleep, so take care to place the urine collection jug in a position that you will notice at night such as near the toilet to remind you. Do not be concerned if small quantities are lost (a quarter cup or 120 mls), but we would ask you to record if you forget and miss a collection. We use urinary creatinine as an indicator of completeness of 24 hour urine collections, but it can vary with body size, muscle mass, and protein intake in young adults.

For a complete 24-hour collection, you must completely empty your bladder in the morning and flush this on the day you choose to make your collection. From this time on (eg., 0700 hours), you will collect all the urine you pass until the same time on the next day (eg. 0700 hours on day two). For this reason, we ask that you record the time you get up to void your bladder on this first day. You need to get up on or just before this time on the next day when you collect all your urine.

Please carefully record the following information on the day that you decide to collect all your urine:

- Date of Collection:_____
- Day of Week: ____Monday, ____Tuesday, ____Wednesday,

  ____Thursday, ____ Friday, ____Saturday, ____Sunday
- _____Time that you rose from bed and completely emptied your bladder, which is considered the time of the start of the collection. <u>Note that you do not save this first morning void</u>; you begin collecting all your urine from this time on.
- _____Time on the following day that you rose from bed and completely emptied your bladder, <u>but this time you collect all of the urine.</u>
- Do you believe that your 24 hour urine collection is complete and that you did not miss collecting any urine? Yes____ No____. If No, can you estimate the volume of urine that you missed collecting?_____

Subject: Office of Science Clearance for use of CFSAN Logo

Date: July 25, 2000

Request:
The attached pages represent the front and back designs proposed for a tee shirt that will be given to the Midshipmen participating in a two- year dietary study at the U.S. Naval Academy. The front design uses the logos of all the participating agencies and advertises that this project is a viable example of several government agencies leveraging their resources to resolve public health issues. We seek approval from the Office of Science for the use of the CFSAN Logo in this application.

We have secured permission and encouragement from Natick's Department of Defense Combat Feeding Program to use their logo and the press office of the Naval Academy informed us that their logos could be used provided the shirts were not sold. We will also seek written approval from the Academy's Human Subject Review Board and other offices as needed.

The Midshipmen are only allowed to wear non-issued apparel if it demonstrates team spirit and support so the right and left sleeves will bare the respective slogans: "Go Navy", and "Beat Army". We believe this incentive will foster a sense of team spirit and belonging to an important study and will encourage compliance with the protocol as it enters its sixth month. This is a common incentive offered to military personnel who, unlike civilians, are not allowed to accept remuneration for participating in longterm studies.

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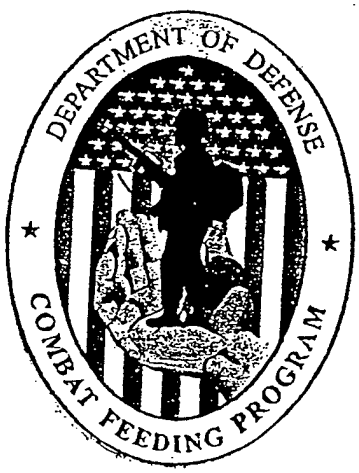
1. Person to contact: Mona S. Calvo, Ph.D. Phone: 205-5199
   Lab: 301-594-5824
2. Title of Project: **IAG # 224-98-2568** A Dietary Strategy to Maximize Bone Mass in United States Naval Academy Midshipmen
3. Study P.I.: Mona S. Calvo, Ph.D.
4. Office and Division: ONPLDS, CRRS
5. If clearance is requested to meet deadline give date: **August 1, 2000**.

6. Supervisor title	Signature	Date
a. Lori A. Love, MD, Ph.D., Director, Clinical Research and Review Staff	Lori A. Love	07/25/00
b. Christine J. Lewis, Ph.D., RD Director, Office of Nutritional Products, Labeling and Dietary Supplements	Christine Lewis PhD	7/28/00
c. Robert L. Buchanan, Ph.D. Senior Science Advisor, Office of Science	Robert L. Buchanan	07/31/00


FDA
CFSAN


UNITED STATES NAVAL ACADEMY
SCIENTIA


DEPARTMENT OF DEFENSE
COMBAT FEEDING PROGRAM

BONE HEALTH

DIETARY SUPPLEMENT STUDY

USNA

★1999 - 2002★

Dietary Supplement Protocol

Study ID # _____ **Team Colors*:** _____

Team Name: _____

***See the attached color-coded key for dietary supplement groups.**

Directions for taking each Supplement:

- **Nutrition Bars:** Eat one (1) per day. You may eat it at any time during the day or night.

- **Calcium Pills:** You will be taking a total of eight (8) capsules per day. Take four (4) in the morning when you wake up and four (4) at night after 1600 hs.
  Remember that you are **not taking an experimental drug and that the capsules contain the same calcium compound as the supplements that you can buy in the grocery store or a starch mixture is used in the placebo or control capsules.

- **Unusual Events:** Please notify us of any changes that you believe may be related to taking either supplements. This would include: bowel changes (constipation or diarrhea) and fatigue or a general feeling of lethargy that continues for more than a week.

- **Compliance:** You are on your honor when reporting your compliance in this study. We will ask you to return any pills that you did not take at the end of every recording period. We will not ask you to return nutrition bars, but we will need to know if you missed any days. Please do not give your bars away (unless they are extra) or trade with another study participant. The recording period will vary depending on the time of year. You are expected to take the bars and the pills each day when you are away from the academy over the summer and on all breaks or during weekend leave. This is necessary in order to know whether or not supplementing your diet for two years is an effective way to build bone and reduce the risk of fracture.

☞ **Contest:** You have been randomly assigned to the color coded Team shown at the top of this page. There are 4 teams and we will track the compliance of each and award prizes periodically throughout the study to the team with the best compliance record. We will post compliance results on the door of office 6B32. Remember you are on your honor and test results will note discrepancies.

☞ **Remember:** We are here to assist you. If you have any questions or concerns at any time, please feel free to contact us. Since I am the study coordinator it is best that you start with me, Sheila Mackertich, R.D. My email address is mackerti@usna.edu and my office number is 3-7929. Please refer to #17 on your consent from for the names and phone numbers of the other contacts in this study.

Abstract submitted to Experimental Biology 2001 meeting in Orlando, FL. March 31-April 4, 2001.
Submitted to the America Society for Nutritional Sciences Topic Category List: Nutrition Metabolism: 5058-ASNS Dietary Assessment

Nutrient Intakes in US Naval Academy Midshipmen (Midn): Comparisons to Dietary Reference Intakes (DRIs) and to National Intakes in Comparable Age Groups. M.S. Calvo, S.K. Mackertich, C.N. Barton, Y.K Park, D.W. Armstrong, R.G. Schultz . CFSAN, FDA, Washington, DC and U.S. Naval Academy, Annapolis, MD.

We assessed nutrient intake status of 1st and 2nd y Midn, (n=173, ♂=112, ♀=61) prior to initiating a 2-y supplement trial. Intake estimates focused on nutrients crucial to late bone growth that are important to maximize peak bone mass and reduce fracture risk. After instruction based on food models, Midn recorded their food intake for 3 consecutive days. Nutrient intakes were determined using FoodProcessor™ and expressed in actual amounts and as a percentage of the DRI. We used the following DRIs in the order of priority: EAR, AI and 1989 RDA or mid-range ESADDI. Intakes were also compared to the *usual* median intakes of comparable groups (17-20 y, ♂=679, ♀=726) from the 1988-94 NHANES III (NH3). Median daily Midn and NH3 intakes in actual amounts and the Midn intakes as percent of the DRI are shown below.

Nutrient, unit	Midn ♂	(%DRI)	Midn ♀	(%DRI)	NH3 ♂	NH3 ♀
Protein, g	114	(197)	72	(156)	92	68
Ca, mg	1335	(120)	936	(82)	916	747
P, mg	1395	(225)	979	(151)	1472	1119
Zn, mg	14.4	(96)	10.0	(83)	12.3	9.7
K, mg	2720	(78)	1882	(54)	2729	2170
Fe, mg	21.1	(209)	15.8	(106)	15.8	12.4
Vit. C, mg	197	(264)	121	(202)	94	82

Median intake of most key bone-building nutrients exceeded those of the Midn's civilian counterparts. For male Midn, median intakes of most nutrients greatly exceeded the DRI. Female Midn also met or exceeded the DRI for many nutrients, but Ca and Zn intakes were only about 80% of the DRI. K intakes of both male and female Midn failed to meet the DRI.

Funded by US Army Medical Research and Materiel Command.